REMARKS

Claims 5, 7-27 and 33-37 are pending after entry of the amendments set forth herein. Claims 1-4, 28-32 and 38-41 are canceled without prejudice. Claims 7, 10, 23 and 33 are amended to correct antecedent support. No new matter is added. Rejections of canceled claims are made moot and will not be further considered. Reconsideration is requested.

Rejections Under §112, ¶1

Claims 1-7, 10-11, 13, 18-19, 23, 28, 31 and 33-37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Office Action states that "the enablement rejection is based on two fundamental enablement problems with the claims. The first problem applies to all of the claims and involves the claims to use a purine or pyrimidine analog having a reactive moiety not normally present in RNA in Claim 1; and a uracil analog having a reactive moiety not normally present in RNA in Claims 5 and 33; to label RNA.

The Examiner asserts "by recitation of such broad language applications are claiming the use of any and all purine or pyrimidine analogs as well as uracil analogs."

Applicants respectfully submit that the present claims under consideration, *i.e.* Claims 5-7, 10-11, 13, 18-19, 23, and 33-37 meet the requirements of 35 U.S.C. 112, first paragraph. The present claims recite the use of a uracil analog in the methods of the invention.

Applicants respectfully submit that the Office's position concerning the scope of the present is misplaced because it fails to adequately allow for a skilled person's knowledge and leaves no room for functional language as a feature of a claim. In the instant case, the claimed subject matter relates to the use of known uracil analogs. Numerous examples of such analogs are known to the skilled person. For example the specification specifically teaches the analogs 2-thiouracil, 4-thiouracil, 2,4-dithiouracil; and further that analogs including sulfonyl, nitro, chloro, bromo, fluoro, sulfamino, aza groups may be used. Applicants note the Examiner's citation of Maddry *et al.* (1978) as prior art for the teaching of "a whole series of purine and pyrimidine analogs".

With respect to the use of such analogs in the methods of the invention, it does not require undue experimentation for one of ordinary skill in the art to select from the available uracil analogs and to perform testing using the methods of the invention to determine which are suitable for use. Such routine optimization is not "inventive, unpredictable and [a] difficult undertaking".

The Examiner cites Melvin *et al.* as evidence of the unpredictability of determining which analogs can be used to label RNA (while also citing the same reference as assertedly making the present invention obvious). However, Applicants note that even in 1978 – 30 years ago – it was simple for one of ordinary skill in the art to obtain various analogs, and test them in cell culture for incorporation. Such methods are not unpredictable given the teachings of the present application.

The Office Action asserts that "the specification does not provide guidance to overcome art recognized problems in determining which precursors would be useful for the methods out of a myriads of purine or pyrimidine precursors that seem to fit the bill". Applicants respectfully submit that the present invention provides a simple and reliable method of biosynthetically labeling RNA. The methods taught by Applicant are straightforward, and can readily be practiced by one of skill in the art. Varying a substrate from among those available does not require immense experimentation, it merely requires minor variation in the experimental methods, such as the courts have recognized is not undue experimentation.

In view of the above remarks, Applicants respectfully submit that the present claims meet the requirements of 35 U.S.C. 112, first paragraph. Withdrawal of the rejection is requested.

Rejections Under §102

Claims 1, 4, 31 and 33 have been rejected under 35 U.S.C. 102(b) as being allegedly anticipated by Melvin *et al.* (1978) Eur. J. Biochem. 92: 373-379 and as evidenced by Woodward et al. (1998) Anyalytical biochemistry 171: 166-172 (both references provided by applicant in IDS).

Applicants respectfully submit that the claimed subject matter is not anticipated by the cited reference. The present claim recites the biosynthetically labeling RNA in a cell of interest by contacting the cell with a uracil analog having a reactive thiol moiety not normally present in RNA, wherein said cell comprises a uracil phosphoribosyltransferase (UPRT).

The Office Action asserts that the cells utilized by Melvin *et al.*, which are hamster kidney cells, comprise a uracil phosphoribosyltransferase, stating that "the fact that the RNA obtained from this cell is labeled with thiol moiety indicated that the cell does contain uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate; wherein said uridine analog is incorporated into RNA synthesized by the cell".

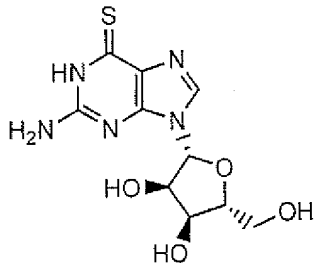
Applicants respectfully submit that it is known in the art and published in refereed scientific journals that mammalian cells do not have UPRT activity, absent its introduction by genetic engineering. The enzyme activity is present in certain protozoa, yeast, bacteria and plants, but not in mammals.

Applicant note, for example, the statement made in Nature Biotechnology, a prestigious, refereed journal, that: "we have developed a method to overcome this limitation by using the salvage enzyme uracil phosphoribosyltransferase (UPRT) from the protozoan *Toxoplasma gondii*. *T. gondii* UPRT has been well-characterized because if its application in monitoring parasite growth: mammals lack this enzyme activity and thus only the parasite incorporates ^{3}H-uracil into its nucleic acids." (Cleary et al. (2005) Nat. Biotech. 23(2):232-7.

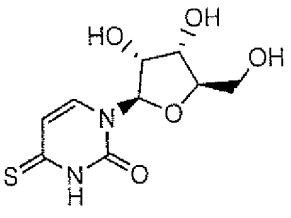
The knowledge of this specificity is based on earlier work, for example by Pfefferkorn *et al.* (1977) J. Parasitology 24:449-453 (previously submitted); by the teachings of Schumacher et al. (2002) PNAS 99:78-83, who found that "The *T. gondii* pyrimidine salvage enzyme uracil phosphoribosyltransferase (UPRT), which is absent in humans, offers a promising target for the design of specific antitoxoplasmal subversive substrates, and several uracil analogues have been identified as substrates of the enzyme".

Applicants respectfully submit that the weight of scientific evidence demonstrates that UPRT enzymatic activity is present in a variety of organisms, but is not present in mammals. Thus, the cited reference does not teach all the limitations of the presently claimed methods.

Applicants further note that the methods of Melvin *et al.* utilize 6-thioguanosine or 4-thiouridine as agents to label RNA. These molecules have the chemical structures as follows:

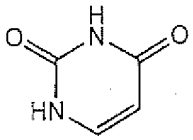


6-thioguanosine



4-thiouridine

In contrast, the methods of the present invention relate to the use of uracil analogs, including 4-thiouracil, which has the structure:



uracil

Applicants note the very different chemistry of uridine and uracil, and respectfully submit that these are not equivalent compounds chemically or biologically. Melvin *et al.* does not teach the use of uracil analogs, including 4-thiouracil or 2,4-dithiouracil.

Withdrawal of the rejection is requested.

Rejections Under §103(a)

Claims 1-2, 5-7, 10-22, 13, 18-19 and 33-34 are rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Melvin *et al.* (1978) Eur. J. Biochem. 92: 373-379 and as evidenced by Woodward et al. (1998) Anyalytical biochemistry 171: 166-172 (both references provided by applicant in IDS) in view of Rana (P.G. Pub 2004/0175732 filed on November 17, 2003 with priority date of November 15, 2002) as evidenced by Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 provided by Applicant in IDS).

Applicants respectfully submit that the cited combination of references does not make obvious the presently claimed invention. As discussed above, Melvin *et al.* fails to teach the fundamental principle of the present application – that a uracil analog can be incorporated into mRNA to provide a specific labeling moiety, through the use of a specific phosphoribyltransferase activity.

The Office Action cites as further evidence the teachings of Woodward *et al.*, which also relate to 6-thioguanosine and 4-thiouridine – molecules that are not uracil analogs, but are rather nucleoside analogs and chemically have very different properties.

Rana et al. is cited as evidence that addition of tags such as biotin, psoralens-biotin, and 4-thiobiotin can be incorporated into RNA. Specifically, reference Is made to paragraph 65, which states:

[0065] In another embodiment where the sequence is known and the interaction between the miRNA and the target RNA is dynamic, biotinylated miRNAs can be made incorporating 4-thio uridine or thymidine or 6-thio guanosine in the miRNA sequence, as illustrated in FIG. 8D. The 4-thio uridine, 4-thio thymidine or 6-thio guanosine can be incorporated into the RNA sequence of the miRNA according to methods known in the art, e.g. as described in Wang. et al. (1998) Biochemistry 37:4243, and Want et al. (1996) Biochemistry 35:6491-6499. The miRNA is transfected into a cell, and miRNA-target RNA complexes are allowed to form. Long wave UV light is used to cross-link the miRNA-target complex, e.g. 350-400 nm, as described in Wang. et al. (1998) Biochemistry 37:4243, and Want et al. (1996) Biochemistry 35:6491-

6499. Total mRNA is isolated from the cell and streptavidin coated beads added to the extract to enrich for the miRNA target complexes. Optionally, the efficiency of crosslinking of miRNA:target complexes can be monitored and optimized according to methods set forth above. Treating the isolated complex with photo-reversible UV 254 generates unmodified (i.e., uncross-linked) RNA species. The complementary DNA form of the miRNA can be added as a primer for subsequent synthesis (RT) reactions. The cDNA primer initiates the synthesis of a full-length sequence complementary to the target RNA by reverse transcriptase (RT) to form a double-stranded complex. The 5' end of the cDNA primer can be extended towards the 3' end of the target RNA using standard methods.

Applicants respectfully submit that the use of biotin as a tag for RNA manipulation is well-known in the art, however the present invention relates to the use of a uracil analog incorporated into RNA to provide a specific labeling moiety, through the use of a specific phosphoribyltransferase activity, which teaching is absent from the cited art (which relates to thiouridine, not a uracil analog).

In view of the above remarks, Applicants respectfully submit that the presently claimed invention is not taught or suggested by the cited art. Withdrawal of the rejection is requested.

Claims 3, 23 and 28 have rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Melvin et al. (1978) in view of Rana (priority date of November 15, 2002) as applied to claims 1 and 2 above further in view of Trudeau et al. (2001) Human Gene Therapy 12:1673-1680.

Applicants respectfully submit that Melvin *et al.* and Rana *et al.* do not teach or suggest the presently claimed invention, as discussed above.

Trudeau does not remedy the deficiencies of the primary references. Trudeau is cited for teaching methods where HGPRT is cloned into a retroviral vector. While the reference teaches the cloning of an enzyme in various permutations, it does not teach a method of contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, where the analog is incorporated into the corresponding nucleotide and wherein the analog is incorporated into RNA comprising the reactive moiety.

Applicants do not dispute that a wide variety of phosphoribosyltransferases, nucleoside kinases and phosphorylases have been cloned and expressed from a wide variety of vectors. However, it is the specific use of the enzymes in Applicants' methods that is claimed, and the sum of the references does not provide essential features of the present invention.

Claims 34-35 and 37 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Melvin et al. (1978) in view of Rana (priority date of November 15, 2002) as applied to

claims 33 and 34 above further in view of Al-Anouti et al. (January 2003) Biochemical and Biophysical Research Communication vol. 302: pp. 316-323.

Applicants respectfully submit that Melvin *et al.* and Rana *et al.* do not teach or suggest the presently claimed invention, as discussed above.

Al-Anouti is cited as a secondary reference against Claims 33-35 and 37. The secondary reference does not remedy the deficiencies of the primary reference. While the reference teaches methods relating to uracil phosphoribosyltransferase, it does not teach a method of contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, where the analog is incorporated into the corresponding nucleotide and wherein the analog is incorporated into RNA comprising the reactive moiety.

Al-Anouti is directed to a method of modulating gene expression of *T. gondii* using dsRNA. In accordance with the methods disclosed by Al-Anouti, a *T. gondii* parasite is transfected with a vector containing the genetic sequence for UPRT, from which dsRNA homologous to UPRT is produced. The dsRNA is degraded into small interfering RNA (siRNA) which then interferes with the UPRT gene so as to down-regulate UPRT gene expression, resulting in decreased UPRT activity. See page 323, 2nd full paragraph. Thus, Al-Anouti proposes a method for decreasing UPRT activity, not for utilizing UPRT activity in the biosynthetic labeling of RNA.

Al-Anouti do not show that a purine or pyrimidine analog can be incorporated into RNA. The reference teaches the use 5-fluoro-2-deoxyuridine (FDUR) for its known toxicity to cells expressing UPRT. However, this toxicity occurs via inhibition of thymidylate synthase (essential for DNA synthesis) by the 5-fUMP produced by the UPRT. There is no evidence in this paper that the 5-fUMP is incorporated into RNA. This does show how UPRT converts a uracil analog to the corresponding uridine monophosphate but not how it converts it to a form that can be used to label RNA.

The engineered expression of UPRT in this reference is designed to express a double stranded RNA construct, not a functional UPRT enzyme. The assertion that the authors designed a plasmid to express UPRT in bacteria and human foreskin fibroblast (HFFs) is not correct. The growth in bacteria is simply for generating plasmid DNA and the construct is never put into HFF cells, the HFF cells are hosts for Toxoplasma that are transfected with the plasmid. There is no transgenic expression of UPRT in an organism other than Toxoplasma in this paper.

Applicants do not dispute that a wide variety of phosphoribosyltransferases, nucleoside kinases and phosphorylases have been cloned and expressed from a wide variety of vectors.

However, it is the specific use of the enzymes in Applicants' methods that is claimed, and the sum of the references does not provide essential features of the present invention.

In view of the above remarks, withdrawal of the rejection is requested.

Claim 36 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Melvin et al. (1978) and Rana (priority date of November 15, 2002) as applied to claim 33 above further in view of Maddry et al. (US Pat. 5,561,225 Oct 1, 1996) as evidenced by Chan (US Pat. 6,403,311 B1 filed Aug 13, 1999) and Iltzsch and Tankersly (1994) Biochem Pharm. Vol. 48 (4): 781-792 cited by applicant in IDS.

Applicants respectfully submit that Melvin *et al.* and Rana *et al.* do not teach or suggest the presently claimed invention, as discussed above.

Maddry *et al.* and Chan teach that 2,4-dithiouracil is a uracil analog; a fact that is not disputed by Applicants, although in combination with the primary references, it does not teach or suggest the presently claimed invention.

The Office Action states that Iltzsch and Tankersley are cited for teaching that 2,4 thiouracil is a substrate for the T gondii enzyme UPRT, and thus 2,4 dithiouracil could be incorporated into RNA using the UPRT enzyme present in the bacterial cell and thiollated RNA can be isolated from such a cell which in turn can be used to conjugate with desired label using the available thiol group in the RNA as a reactive moiety.

Applicants respectfully submit that Iltzsch and Tankersley fail to teach methods wherein cells are contacted with a purine or pyrimidine analog. All of the work identifying UPRT substrates by Iltzsch and Tankersley were performed using parasite lysates, not living organisms outside the animal. There is no guarantee that a live cell will efficiently take up a given substrate, let alone that that will be a substrate for subsequent steps in the pathway (i.e., the kinase that takes UMP to UTP or the RNA polymerase that incorporates that into RNA) or be bioavailable in an animal.

There is also no evidence in Iltzsch and Tankersley that any of the uridine monophosphate forms of these analogs will be substrates for RNA polymerase and end up incorporated into RNA. So there is no evidence in this paper to show that addition of uracil analogs to whole cells will result in labeling of RNA.

Applicants respectfully submit that the present claims meet the requirements of 35 U.S.C. 103(a). Withdrawal of the rejection is requested.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-304.

Respectfully submitted,
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Date: March 6, 2008

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